Mechanical relaxation of the hair bundle mediates adaptation in mechanoelectrical transduction by the bullfrog's saccular hair cell

(sacculus/sensory adaptation)

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ABSTRACT   Mechanoelectrical transduction by hair cells of the frog's internal ear displays adaptation: the electrical response to a maintained deflection of the hair bundle declines over a period of tens of milliseconds. We investigated the role of mechanics in adaptation by measuring changes in hair-bundle stiffness following the application of force stimuli. Following step stimulation with a glass fiber, the hair bundle of a saccular hair cell initially had a stiffness of ≈ 1 mN·m^{-1}. The stiffness then declined to a steady-state level near 0.6 mN·m^{-1} with a time course comparable to that of adaptation in the receptor current. The hair bundle may be modeled as the parallel combination of a spring, which represents the rotational stiffness of the stereocilia, and a series spring and dashpot, which respectively, represent the elastic element responsible for channel gating and the apparatus for adaptation.

The hair cell is a mechanoreceptor: whether it is ultimately stimulated by sound, acceleration, or substrate motion, every hair cell transduces mechanical stimuli into electrical responses. Although stimuli reach hair cells in various organs of the internal ear and in the lateral-line system by diverse routes, the response of each hair cell is initiated by the application of a force to its mechanoreceptive organelle, the hair bundle. The resulting deflection of the bundle's tens to hundreds of stereocilia somehow excites an electrical response in the hair cell (1).

Because the hair cell's response ensues from a mechanical input, an understanding of the transduction process requires the definition of the forces acting on and originating within the hair bundle. As an initial step toward such a description, several laboratories have measured the steady-state stiffnesses of hair bundles by use of the force-fiber technique (2–4). If a fine flexible glass fiber is attached to a hair bundle's distal tip and subsequently moved, the bundle is deflected in proportion to the static force exerted against it. The dependence of this response on the height at which force is exerted against the bundle indicates that the bundle behaves primarily like a rigid rod attached to a basal pivot (3, 4).

Although displacement of the hair bundle's distal tip excites mechanoelectrical transduction (5), it is still uncertain how this motion gates the opening and closing of transduction channels. Some of the work that is done in displacing the bundle must be performed on the channels themselves (6). Do the channels sense the flexion of the stereocilia directly, or are they gated by elastic elements that are stressed by internal rearrangements within the bundle (6–8)?

We have made use of adaptation in the transduction process of hair cells from the bullfrog's sacculus to measure a mechanical component of the hair bundle that is involved in the gating of transduction channels. The bullfrog's saccu-lus, a detector of vibrations in the frequency range 10–200 Hz (9), has afferent-fiber thresholds corresponding to substrate accelerations as low as 100 μm·s^{-2} (10). This sensitivity is remarkable because the sacculus constantly experiences the static acceleration due to gravity, nearly 10 m·s^{-2}. Although the mechanics of the whole saccular organ may act to filter some static accelerational inputs prior to the site of transduction (11), the sacculus also rejects static inputs by means of an adaptation process in its hair cells (12, 13). This process continuously resets the transducer's position of maximal sensitivity to compensate for static hair-bundle deflections.

We have asked whether adaptation of the mechanoelectrical transducer is associated with mechanical changes in the hair bundle. To answer this question, we have refined the force-fiber technique so as to measure displacements of a hair bundle with a spatial resolution of <1 nm and a temporal resolution of <100 μs. We find that the hair bundle undergoes a mechanical relaxation that is correlated in time course, polarity, and direction with the simultaneously measured adaptation of the mechanoelectrical transduction current (14). We believe that this mechanical relaxation is a consequence of a hitherto unobserved elastic element whose existence was predicted on the basis of response kinetics (7) and whose putative function is to gate the transduction channel.

METHODS

Preparation. Experiments were performed on the isolated saccular epithelium of the bullfrog, *Rana catesbeiana* (5). After removal from the inner ear, the saccular macula was treated with subtilopeptidase BPN' (50 mg/liter) (Sigma) in frog saline solution (110 mM Na$^+$/2 mM K$^+$/4 mM Ca^{2+}/118 mM Cl$^-$/3 mM D-glucose/5 mM Hepes buffer, pH adjusted to 7.25) for 40 min to facilitate the subsequent removal of the otolithic membrane. The macula was securely pinned to the bottom of a 500-μl chamber and visualized under bright-field optics with a ×40, water-immersion objective lens of numerical aperture 0.75 (Carl Zeiss, Oberkochen, F.R.G.). This preparation allows direct access to the hair bundles protruding from the apical surfaces of the hair cells (Fig. 1). All experiments were performed at room temperature (18°C–22°C) on large hair cells at the abneural margin of the sacculus.

Microelectrode Recording. Microelectrodes were bent 0.5–1.0 mm from their tips and filled with 3 M KCl; their tips (resistance, 100–300 MΩ) were inserted into the apical surfaces of hair cells with the aid of Huxley-type micromanipulators (Frederick Haer, Brunswick, ME). Recordings were made with DC amplifiers and a simple voltage-clamp circuit (15); the cells' input resistances were 200–1000 MΩ at the holding potential of −70 mV and the membrane capacitances were $\approx$20 pF. To increase the recording bandwidth, the electrodes were coated with silicone plastic (Sylgard 184, Dow) to within 0.5–1.0 mm of their tips. Membrane current, measured using a current-to-voltage

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converter connected to the bath's ground electrode via an agar bridge, was filtered at 500 Hz with an eight-pole Bessel filter. Data were digitized on-line and analysis was performed on a PDP-11/23 computer (Digital Equipment, Maynard, MA) running BASIC-23 (Indec Systems, Sunnyvale, CA).

Force-Fiber Technique. In a force-fiber measurement of a hair bundle's stiffness, the tip of a flexible, horizontally mounted glass fiber is attached to the hair bundle (Fig. 1) and the fiber's base is moved horizontally at a right angle to the fiber's axis. If the fiber's distal tip (and the adherent hair bundle) moves by a distance x when the fiber's base is displaced by a distance y, the force applied to the bundle is given by

$$F = \kappa_F(y - x),$$

in which κ_F is the stiffness of the fiber's tip (3). When attached to the bulbous tips of kinocilia, the fibers used in these experiments were typically 2–10 times as flexible as the hair bundles. As a consequence, a displacement step applied to the base of a fiber resulted in a force stimulus that was nearly constant but that diminished by 5–16% while the bundle was relaxing. This modest diminution deemphasizes the mechanical relaxation of the hair bundle during adaptation.

The dimensions of the fibers were determined by the temporal and spatial resolution required in the experiments. The usefulness of a fiber in monitoring bundle stiffness depends on its being bent measurably during the application of a small force; it is therefore desirable that the compliance at the fiber's tip be larger than that of the hair bundle. On the other hand, to move the bundle rapidly, the fiber should experience less hydrodynamic damping than does the bundle. Hydrodynamic drag increases with increasing length and diameter of the fiber, while compliance increases with length but decreases with increasing diameter. Hence, by making a fiber's diameter sufficiently small, a high compliance can be achieved with a fiber of arbitrarily small length and drag. In this way, it is possible to make a fiber whose drag does not materially slow the motion of the bundle, but that is sufficiently flexible to be responsive to the minute forces exerted by a hair bundle.

Fibers ≈250 nm in diameter were formed from borosilicate glass (Kimble KG-33, Garner Glass, Claremont, CA), mounted at their bases upon thicker glass shanks, and cut to lengths of ≈100 μm. To increase their optical contrast, the fibers were sputter-coated with an ≈100-nm layer of gold-palladium
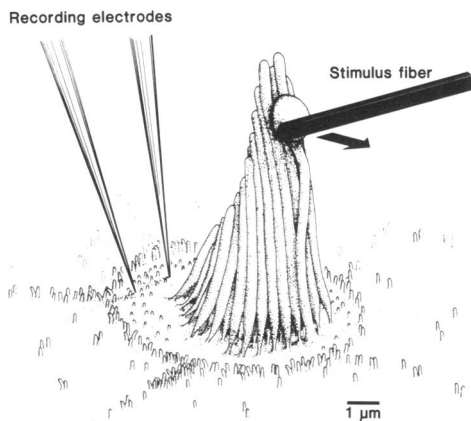

FIG. 1. Diagram of the apical surface of a saccular hair cell. The tip of the gold-palladium-coated fiber was coupled to the kinociliary bulb; movement of the base of the flexible fiber forced the hair bundle to undergo a displacement. Positive stimulus direction (16) is indicated by the arrow. Electrodes inserted into the apical surface of the hair cell measured the receptor current resulting from the displacement of the bundle.
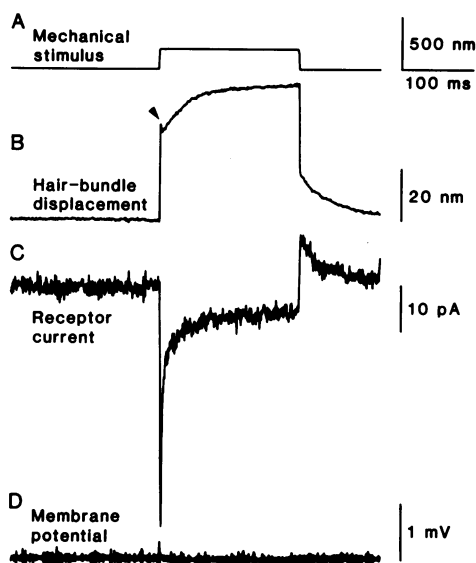
(Hummer VI, Anatech, Alexandria, VA). The stiffness of each fiber was calibrated with an accuracy of 10% by measuring the vertical deflection ensuing from attaching to its distal tip an acrylic sphere of known size and density; stiffnesses were in the range 80–350 μN·m⁻¹. The stiffnesses of most fibers were also inferred from measurements of the Brownian motion of their tips in saline solution (3); the results agreed with those obtained by the direct method. When immersed in saline solution and attached to hair bundles, fibers of these dimensions displayed a flat frequency response up to a corner frequency of ≈300 Hz, as determined from their step responses.

The shank of a fiber was held in a piezoelectrical micromanipulator, which also served to move the fiber's base through calibrated displacements (17). To prevent excitation of the stimulator's resonance at 2 kHz, the driving signal was filtered at 800 Hz with a 16-pole Bessel filter; the manipulator's frequency response was then flat to 1 kHz.

The image of the tip of the fiber (×1000 magnified) was projected onto a pair of photodiodes (UV-140-2, EG&G Electro-optics, Salem, MA). Each photodiode's current was proportional to the intensity of the illuminating light, which was supplied by a 100-W tungsten bulb with a stabilized dc power supply. The photocurrents were converted to voltages and the differential voltage was proportional to the position of the fiber over a distance equal to the fiber's optical diameter of 0.5–1 μm. The time constant of the detector's circuitry, ≈100 μs, was set by the current-to-voltage converter's 100-MΩ feedback resistor and stray capacitance.

Displacement Calibration. Two methods were used to calibrate the output of the photodiode headstage. In the first, the freely moving probe was displaced a known distance using the piezoelectrical stimulator, and the voltage output of the photodiode headstage was recorded. In the second, the photodiodes were mounted on a bimorph and stepped 10 μm in the image plane (18). Due to the optical magnification factor of ×1000, this calibration step corresponded to a 10-nm step in the object plane. The calibration methods were in agreement.

The resolution of measurements was limited by photon shot noise in the illuminating source and by acoustical and ground-borne vibrations in the recording environment. By placing the recording apparatus on a vibration-isolation table (GS-34-FR, Newport, Fountain Valley, CA) immediately above bedrock, we achieved a root-mean-square noise at the fiber's tip of 200 pm over the frequency band 7–1000 Hz.

RESULTS

Mechanical Relaxation. The stiffnesses of individual hair bundles from the bullfrog's sacculus were assayed by using calibrated flexible glass fibers to apply known forces to the bundles. When the tip of a fiber was brought into contact with a hair bundle, it usually stuck tightly enough to allow the bundle to be pulled as well as pushed. In most experiments, the fiber was attached to the bulbous swelling at the tip of the kinocilium (Fig. 1), the single true cilium in the hair bundle and the site of mechanical stimulation *in vivo*.

When the base of the fiber was moved in a stepwise manner, the force stimulus produced a rapid initial displacement of the hair bundle; the displacement then increased exponentially with a time constant of 33 ms (Fig. 2B). The hair bundle was initially ≈75% stiffer than it became in the steady state. In a sample of 34 hair bundles, the mean steady-state or static stiffness was 0.63 ± 0.22 mN·m⁻¹ (SD; $n = 34$) compared to a dynamic stiffness of 1.00 ± 0.42 mN·m⁻¹ (SD; $n = 34$). The time constant of the relaxation depended on the polarity and magnitude of the applied force. At the onset of small positive displacements of <50 nm, the mean time constant for relaxation was 27 ± 17 ms (SD; $n = $

FIG. 2. Simultaneous mechanical and receptor–current responses to a force step. (A) The base of the fiber was given a 186-nm positive step displacement by a piezoelectrical micromanipulator. (B) The bundle underwent an abrupt initial displacement followed by a slow relaxation. The notch in the bundle displacement (arrowhead) was seen in many instances. (C) The current measured simultaneously with a two-electrode voltage clamp had an initial, fast, inward transient followed by a slower adaptation. An overshoot and slow adaptation occurred at the end of the step. The two components of adaptation in the receptor current had time courses similar to those of the mechanical relaxations. (D) The cell's membrane potential was effectively clamped at -70 mV. The stiffness of the force fiber was 340 μN·m^{-1}.

34; range, 7–80 ms); the time constant for relaxation following the termination of such steps was 32 ± 16 ms (SD; $n = 34$). The time constant at the stimulus' onset decreased by up to a factor of 2 for large (>200 nm) steps in the positive stimulus direction; the use of large negative stimuli was associated with a 2-fold increase in this time constant. A small notch was often observed following the initial rising phase of the displacement to positive steps (Fig. 2B, arrowhead).

Transduction Current. The time constant of the mechanical relaxation was similar to that reported for adaptation of receptor currents in hair cells of the bullfrog's sacculus (12, 13). To relate the two phenomena, we used a two-electrode voltage-clamp circuit to record the transduction current during responses to stimulus steps. A small positive deflection of the hair bundle did not elicit a static receptor current (Fig. 2C); instead, after a brief transient, the response declined exponentially from an amplitude of -15 pA to a steady-state level of only -5 pA. Following the cessation of the step, there was an 8-pA overshoot of the resting current, toward which the response then relaxed exponentially.

Both of the relaxations in receptor current, which we shall term adaptation, had time courses similar to those of the mechanical relaxations. The time constant of receptor-current adaptation at the onset of small positive stimulus steps was 27 ± 14 ms (SD; $n = 11$); the time constant of adaptation following the termination of such steps was 35 ± 27 ms (SD; $n = 11$). In four cells from which simultaneous mechanical and current measurements were made, the time constant of the current adaptation at the step's conclusion was highly correlated ($r > 0.99$) with the time constant of the concurrent mechanical relaxation. The regression slope, 0.88 ± 0.04 (SEM), was not significantly different from unity by a t test.

Similar results were obtained with single-electrode recordings of receptor potentials. Each cell was hyperpolarized to

around -80 mV into the linear, high-impedence region of its current–voltage relation (19). The cell's membrane time constant was measured by injecting current steps. The receptor potentials recorded in response to force steps were then compensated for this time constant; the resultant waveforms represent membrane current. The results from eight cells resembled those from voltage-clamp recordings: a rapid transient in receptor current was followed by two phases of adaptation that correlated with the simultaneously measured mechanical relaxations.

Positive-going deflection of a hair bundle often elicited a large inward-current transient (Fig. 2C). This component of the response, whose duration of 1–2 ms was at the limit of our recording bandwidth, coincided with the notch in the recording of bundle position (Fig. 2B). For bundles displaced in the positive direction by <100 nm, the mean sensitivities of mechanoelectrical transduction during the initial transient, the steady-state response, and the overshoot were, respectively, -1.39 ± 0.31 mA·m^{-1}, -0.15 ± 0.09 mA·m^{-1}, and 0.22 ± 0.08 mA·m^{-1} (SD; $n = 11$).

Directionality. Transduction channels only open when the hair bundle's displacement has a component in the positive stimulus direction (16). To ascertain whether the mechanical relaxation is related to transduction, we measured the stiffness of hair bundles in this direction and then, after rotating the microscope stage through 90°, in the orthogonal direction. The mechanical relaxation was markedly reduced for stimuli oriented in the orthogonal direction (Fig. 3). In four experiments, the bundle was subsequently rotated back to its original orientation, whereupon mechanical relaxation reappeared. When measured at the level of the kinociliary bulb, the orthogonal stiffness was significantly less than the parallel stiffness: the ratios of the orthogonal stiffness to the dynamic and static parallel stiffnesses were, respectively, 0.59 ± 0.08 (SEM; $n = 8$; $P < 0.002$) and 0.81 ± 0.09 (SEM; $n = 8$; $P < 0.1$).

Kinocilium. The mechanical relaxation was caused neither by the coupling of the probe to the kinocilium nor by the coupling of the kinocilium to the rest of the hair bundle. Identical relaxations were observed when the fiber was placed against the short edge of the bundle, opposite the kinocilium. The relaxation also persisted when the kinocilium was surgically removed. Following this procedure (20), the relaxation became smaller and slower, perhaps due to damage to the cell.

Other Controls. The measurements in the orthogonal direction showed that the relaxation is not an artifact of the stimulating or recording system. Other observations support this view. The mechanical relaxation and the current adaptation were physiologically labile: both processes became, hand-in-hand, smaller and slower with time. The relaxation also persisted when the extracellular calcium concentration was reduced from 4 mM to 250 μM, which is roughly the value that the bundle normally faces *in vivo* (A.J.H. and R.
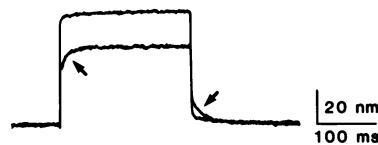

FIG. 3. Directional character of the mechanical relaxation. The stiffness of this hair bundle was first measured parallel to the bundle's plane of morphological symmetry; the cell was then rotated 90° with the microscope stage and the stiffness was measured in the orthogonal direction. The mechanical relaxation observed upon stimulation in the parallel direction (arrows) was not seen for stimulation in the orthogonal direction. The displacement in the orthogonal direction was greater than that in the parallel direction, showing that the bundle was less stiff along the orthogonal axis.

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Jacobs, unpublished data). No slow relaxation was observed in the movement of the unengaged probes. Moving an artificial hair bundle, of similar stiffness to an actual bundle but made from a glass fiber, revealed no slow relaxation. Finally, the Brownian motion of the hair bundle along its axis of morphological symmetry had a low-frequency component that was consistent with the slow relaxation process (21).

Mechanical Resonances. An earlier study (3) found both spontaneous oscillation and force-induced resonance of hair bundles in the turtle's cochlea. Of the hundreds of cells tested in the present study, only two displayed such behavior (Fig. 4). Mechanical resonance was not a prerequisite for current-induced electrical resonance, spontaneous electrical resonance, or sensitive transduction, all of which were present in saccular hair cells that did not oscillate or resonate mechanically. In the two cells, the mechanical resonance was superimposed on the slower mechanical relaxations of the hair bundle; the two processes are thus not mutually exclusive.

DISCUSSION

Our principal finding is that the hair bundle of the bullfrog's saccular hair cell is not mechanically simple, but includes at least two distinct compliant elements and one damping element. One minimal phenomenological model consistent with the observed mechanical properties of the bundle is a Maxwell element: a spring in series with a dashpot, both of which are in parallel with another spring (Fig. 5A). Following a step force, this network is initially quite stiff because the two springs are in parallel; over time, however, the dashpot relieves the tension in the spring in series, making the network more compliant. The stiffness of the series spring, which equals the difference between the dynamic and static stiffnesses, is ≈ 0.4 mN·m^{-1} or 60% the stiffness of the parallel spring. As calculated from the time constant of the mechanical relaxation, the damping by the dashpot is ≈ 6 μN·s·m^{-1}.

The mechanical relaxation of the hair bundle is associated in two ways with adaptation of the mechanoelectrical transduction process. First, both the relaxation and transduction occur for stimuli that lie within the hair bundle's plane of mirror symmetry but do not ensue from orthogonal stimulation. Second, the time courses of mechanical relaxation and of response adaptation are quite similar. It is simplest to suppose that mechanical relaxation in the hair bundle mediates adaptation of the transducer. The polarity of the mechanical relaxation suggests that adaptation to a positive stimulus results from reduction in the tension in an elastic element that gates the transduction channel. We accordingly propose that the spring in series with the dashpot gates the
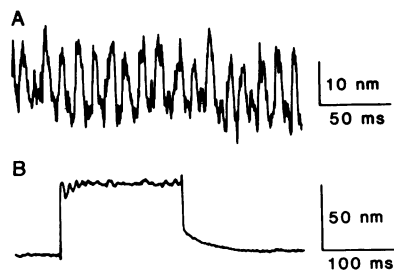

FIG. 4. Mechanical resonance of a hair bundle. (A) The tip of the hair bundle of this cell underwent spontaneous oscillation at 80 Hz. (B) The displacement of the bundle in response to a force step displayed a damped resonance following the onset of the step (average of 16 traces; note differences in scales). Oscillation is superimposed on a slower relaxation, seen more clearly following the end of the step. Of >100 cells tested in this study, only this and one other cell showed mechanical resonance.
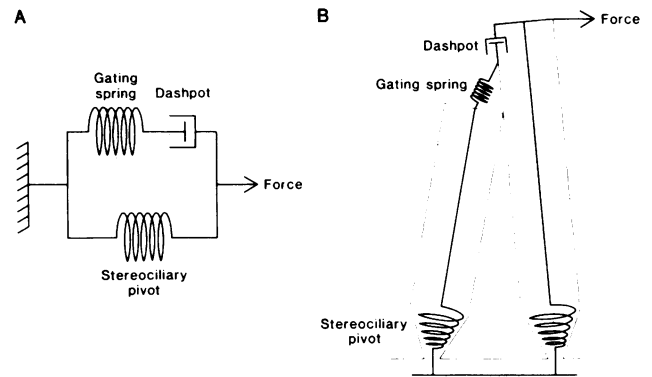

FIG. 5. Mechanical models of the saccular hair bundle. (A) The Maxwell element is a phenomenological model whose behavior mimics the mechanical response of the bundle. We attribute one spring to the stereociliary pivot and the other to an elastic element that gates the opening and closing of the transduction channel; the dashpot represents a mechanical element that relaxes during adaptation. (B) A model in which the mechanical elements of A have been superimposed on a simplified representation of the hair bundle. The transduction channels are thought to lie at one or both ends of the gating spring and to be gated by tension in that spring. Note that during stimulation the gating spring elongates but the pivotal springs bend.

transduction channels, and we term this elastic element the gating spring. The existence of such an elastic element was postulated to explain the response kinetics of saccular hair cells (7) and the Boltzmann relation between steady-state displacement and transduction current (8).

We have two reasons to equate the spring in parallel with the gating spring with the rotational stiffness of the stereocilia at their insertions into the cellular apex, the stereociliary pivots. First, stereocilia have a core of filamentous actin that extends through their basal tapers (22); calculations indicate that the pivotal stiffness of this actin fascicle should be comparable to the measured stiffness of the hair bundle (3, 4). Second, individual stereocilia are radially symmetrical and hence should be equally stiff when bent in any direction; the smaller, nonrelaxing stiffness measured when the bundle is displaced in the direction orthogonal to its plane of symmetry is interpreted as being due to the stereociliary pivots alone.

The static stiffness of hair bundles in the bullfrog's sacculus was typically 0.6 mN·m^{-1} for either positive or negative stimulation in the bundle's plane of symmetry. This stiffness is similar to those of hair bundles in the sacculus of another frog (4) and in the cochleas of the turtle (3) and guinea pig (2). When measured using a two-electrode voltage clamp, the sensitivity of the transducer of bullfrog saccular hair cells was around 1 mA·m^{-1}. This sensitivity is comparable to that measured in tight-seal whole-cell recordings from hair cells of the frog's sacculus (8) and turtle's cochlea (23), and somewhat higher than that reported for hair cells of the chicken's labyrinth (24). Unlike the present and earlier studies of the bullfrog's saccular hair cells (12), recordings made with tight-seal electrodes showed no adaptation to maintained displacement (8). Two factors may explain why different techniques gave dissimilar results on the same hair cells: the relatively large tight-seal electrode may impede mechanical rearrangements within the cuticular plate against which the electrode presses, or some cytosolic factor may be lost or buffered during the dialysis of the hair cell (8). Our results do confirm and extend the observation of adaptation in response to displacements of, rather than constant forces on, the hair bundle (12, 13). The fast-transient component was probably not observed in previous intracellular recordings because the displacement steps had rise times of 10 ms, which is much slower than the transient. The decline of the fast-transient

component could be due to a rapid reduction either in the conductance of the transduction channels or in the probability of their being in the open state; at present, we cannot distinguish between these possibilities.

The minimal mechanical model is doubtlessly an oversimplification. Because adaptation is generally incomplete (12, 13), the gating spring has a nonadapting component that is in parallel with the dashpot. If the incomplete character of adaptation is taken into account, the stiffness of the gating spring is more nearly equal to that of the parallel spring. The other simple mechanical model consistent with the bundle's measured behavior is a Voigt element: a spring with a parallel dashpot, both in series with a second spring. This model does not, however, offer a ready explanation for either adaptation of the receptor current or the relative bundle stiffnesses for stimuli in the plane of symmetry and for orthogonal ones.

The dashpot included in the passive linear model might, together with the gating spring, correspond to a single viscoelastic element. Several arguments suggest, however, that the dashpot is a simplification of a more complex apparatus. Adaptation is metabolically labile (13) and hence unlikely to be entirely passive. Because its rate depends on stimulus polarity, adaptation is a nonlinear process. The relaxation of some hair bundles was not monotonic, but transiently overshot the steady-state value. The notch often seen on the rising phase of bundle-displacement records and the mechanical resonance observed in two cells also suggest that the hair bundle has both passive and active elements. Finally, it seems unlikely that the stereocilia harbor a spring-like element that operates in compression.

One speculative mechanism for mechanical relaxation of the hair bundle is illustrated (Fig. 5B) in a superposition of the elements of the mechanical model onto the morphological components of the hair bundle (1); for the sake of concreteness, we have identified the gating spring with a filamentous link between adjacent stereocilia (25). We suppose in this model that the dashpot corresponds to a constitutively active force generator, which, when in equilibrium, supports a resting tension in the gating spring. The steady-state proportion of open transduction channels would be set by the resting tension in this spring. After a positive displacement, the increased tension in the gating spring is gradually relieved as the spring's attachment to the neighboring stereocilium via the force generator is dragged down to a new equilibrium position. After a negative displacement, the force generator must crawl up the stereocilium to restore the resting tension. That the mechanical relaxation becomes faster for steps in the positive direction and slower for steps in the negative direction is consistent with this model: tension in the gating spring would, in a natural way, be expected to have such an effect on the rate constants of the force generator (26). An active mechanical element need not reside in the hair bundle itself. Because the geometrical relationships among stereocilia are also determined by the structure into which the stereocilia insert, it is possible that mechanical relaxation of the bundle instead ensues from changes in the shape of the cuticular plate (27).

The existence of a force-generating process at the apex of the hair cell is not implausible. The cuticular plate contains actin, myosin, and α-actinin (28). In addition to actin, stereocilia may contain myosin (29) and an actin-binding 110-kDa ATPase (30). The maximum rate of shear between the tips of stereocilia during relaxation is of the order of 1 μm·s^{-1}, similar to the maximum velocity of myosin heads relative to actin filaments in muscle (31). If one force generator were associated with each of the hair bundle's tens to hundreds of transduction channels (8, 24), each would need to provide a force comparable to that of an actin–myosin reaction (26, 31).

Perhaps the most interesting conclusion to be drawn from these experiments is that displacement of the hair bundle's tip is not strictly the stimulus for mechanoelectrical transduction. This follows from the observation (Fig. 2) that, even though the bundle is moving in the "excitatory" direction for several tens of milliseconds after the onset of a positive force step, the inward transduction current is actually decreasing! We propose that the proximate excitatory stimulus to the transduction apparatus is instead tension in a specific elastic element, the gating spring.

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